

Ligation

Requirements:

- Digested DNA
- T4 ligase and buffer (produced by Takara Bio®)
- Nuclease-free 0.2mL PCR tubes
- Water bath at 16°C or fridge at 4°C

Protocol:

1. Prepare reaction systems in a 0.2mL PCR tube according to the following table:

Components	Volume (uL)
Insert	V_1
Vector	V_2
Buffer	1μL
T4 Ligase	1μL

$$\frac{V_1}{V_2} = \frac{3 \times M_1 \times C_2}{1 \times M_2 \times C_1}$$

V_1 : insert

V_2 :vector

M: molar weight (size)

C: conc. (ng/mL)

2. Incubate in the water bath at 16°C for 6 hours or fridge at 4°C for 12 hours.